

1 What is claimed is:

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4 1. A recombinant immunotoxin polypeptide and pharmaceutically  
5 acceptable salts thereof comprising a CD3-binding domain and a  
6 *Pseudomonas* exotoxin (PE) mutant, said PE mutant having ADP-  
7 ribosylating and translocation functions but substantially  
8 diminished cell-binding ability.

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10 2. A recombinant immunotoxin polypeptide and pharmaceutically  
11 acceptable salts thereof according to claim 1 wherein the CD3-  
12 binding domain comprises an anti-CD3 antibody or CD3-binding  
13 fragment thereof.

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15 3. A recombinant immunotoxin polypeptide and  
16 pharmaceutically acceptable salts thereof according to claim 2  
17 wherein the anti-CD3 antibody or CD3-binding fragment thereof  
18 binds an epitope on the  $\epsilon$  chain of human CD3.

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20 4. A recombinant immunotoxin polypeptide and pharmaceutically  
21 acceptable salts thereof according to claim 2 wherein the anti-  
22 CD3 antibody or CD3-binding fragment thereof binds an epitope  
23 formed by the  $\epsilon$  and  $\gamma$  chains of human CD3.

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25 5. A recombinant immunotoxin polypeptide and pharmaceutically  
26 acceptable salts thereof according to claim 2 wherein the CD3-  
27 binding domain comprises a Fab fragment of an anti-CD3 antibody.

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29 6. A recombinant immunotoxin polypeptide and pharmaceutically  
30 acceptable salts thereof according to claim 2 wherein the CD3-  
31 binding domain comprises the Fv region, or a CD3-binding  
32 fragment thereof, of an anti-CD3 antibody.

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1 7. A recombinant immunotoxin polypeptide and pharmaceutically  
2 acceptable salts thereof according to claim 2 wherein the CD3-  
3 binding domain comprises monoclonal antibody UCHT-1 or a CD3-  
4 binding fragment thereof.

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6 8. A recombinant immunotoxin polypeptide polypeptide and  
7 pharmaceutically acceptable salts thereof according to claim 2  
8 wherein the CD3-binding domain comprises (the Fv region,) or a  
9 CD3-binding fragment thereof, of an antibody selected from:  
10 monoclonal antibody UCHT-1, an antibody having a variable region  
11 which is at least 80% identical to the variable region of UCHT-  
12 1, an antibody having complementarity-determining regions  
13 identical with those of UCHT-1 and having at least one sequence  
14 segment of at least five amino acids of human origin, and an  
15 antibody competing with UCHT-1 for binding to human CD3 antigen  
16 at least about 80% as effectively on a molar basis, and having  
17 at least one sequence segment of at least five amino acids of  
18 human origin.

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20 9. A recombinant immunotoxin polypeptide and pharmaceutically  
21 acceptable salts thereof according to claim 2 wherein the CD3-  
22 binding domain comprises a single chain Fv of an anti-CD3  
23 antibody.

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25 10. A recombinant immunotoxin polypeptide and pharmaceutically  
26 acceptable salts thereof according to claim 8 wherein (the Fv)  
27 region is a single chain Fv.

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29 11. A recombinant immunotoxin polypeptide and pharmaceutically  
30 acceptable salts thereof according to claim 10 wherein the CD3-  
31 binding domain comprises a single chain Fv of UCHT-1.

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2 12. A recombinant immunotoxin polypeptide and pharmaceutically  
3 acceptable salts thereof according to claim 1 comprising a  
4 single chain Fv of UCHT-1 fused to a PE mutant essentially  
5 deleted of its cell-binding domain.

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7 13. A recombinant immunotoxin polypeptide and pharmaceutically  
8 acceptable salts thereof according to claim 12 wherein the PE  
9 mutant is PE38.

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11 14. A recombinant immunotoxin polypeptide and pharmaceutically  
12 acceptable salts thereof according to claim 1 consisting  
13 essentially of the single chain Fv of an anti-human CD3 antibody  
14 fused via the carboxy terminus thereof to a PE mutant  
15 essentially deleted of its cell-binding domain.

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17 15. A recombinant immunotoxin polypeptide and pharmaceutically  
18 acceptable salts thereof according to claim 14 having the  
19 formula  $V_L - L - V_H - C - PE$  mutant.

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21 16. A recombinant immunotoxin polypeptide and pharmaceutically  
22 acceptable salts thereof according to claim 15 wherein  $V_L$  and  $V_H$   
23 are derived from UCHT-1 and the PE mutant is PE38.

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25 17. A recombinant immunotoxin polypeptide selected from  
26 polypeptides having residues 1-601, 2-601 and 3-601 of Sequence  
27 ID. NO: 1, homologs of said polypeptides which are at least 80%  
28 identical thereto and their pharmaceutically acceptable salts.

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30 18. A recombinant immunotoxin polypeptide according to claim 17  
31 having residues 3-601 of SEQ. ID No:1 and its pharmaceutically  
32 acceptable salts.

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1 19. A nucleic acid molecule encoding the recombinant  
2 immunotoxin polypeptide of claim 1.

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4 20. A method of preparing a recombinant immunotoxin polypeptide  
5 of claim 1.

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7 21. A method for treatment or prophylaxis of T-cell mediated  
8 disorders in a patient comprising administering to a patient in  
9 need thereof a therapeutically effective amount of a recombinant  
10 immunotoxin polypeptide or its pharmaceutically acceptable salt  
11 according to claim 1.

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13 22. A method for treatment or prophylaxis of organ  
14 transplantation rejection in a transplant patient comprising  
15 administering to the patient a therapeutically effective amount  
16 of a recombinant immunotoxin polypeptide or its pharmaceutically  
17 acceptable salt according to claim 1.

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19 23. A method for treatment or prophylaxis of autoimmune disease  
20 in a patient comprising administering to the patient a  
21 therapeutically effective amount of a recombinant immunotoxin  
22 polypeptide or its pharmaceutically acceptable salt according to  
23 claim 1.

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25 24. An autologous therapy for treating or preventing a T-cell  
26 mediated disorder or condition in a patient, comprising:

27 (a) recruiting from the patient a cell population  
28 comprising CD3-bearing cells;

29 (b) treating the cell population with a recombinant  
30 immunotoxin polypeptide or its pharmaceutically acceptable salt  
31 according to claim 1 to at least partially deplete said cell  
32 population of CD3-bearing cells; and

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1 (c) reinfusing the treated cell population into the  
2 patient.

4 25. A method for treatment or prophylaxis against graft versus  
5 host disease in patient to undergo a bone marrow transplant  
6 comprising:

7 (a) providing an inoculum comprising isolated bone marrow  
8 and/or stem cell-enriched peripheral blood cells of a suitable  
9 donor treated with a T-cell depleting effective amount of a  
10 recombinant immunotoxin polypeptide or its pharmaceutically  
11 acceptable salt according to claim 1; and

12 (b) transplanting the inoculum into the patient.

14 26. A method for the treatment or prophylaxis or treatment of  
15 transplant rejection in a patient to undergo a bone marrow  
16 transplant comprising:

17 (a) reducing the levels of viable CD3-bearing cell  
18 population in the patient;

19 (b) providing an inoculum comprising isolated bone marrow  
20 and/or stem cell-enriched peripheral blood cells of a suitable  
21 donor treated with a T-cell depleting effective amount of a  
22 recombinant immunotoxin polypeptide or its pharmaceutically  
23 acceptable salt according to claim 1; and

24 (c) introducing the inoculum into the patient, and  
25 thereafter optionally administering a recombinant immunotoxin  
26 polypeptide according to claim 1 to the patient to further  
27 deplete donor and patient T cells.

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29 27. A method of conditioning a patient to be transplanted with  
30 cells, or a tissue or organ of a donor, the method comprising:

31 (a) depleting the CD3-bearing cell population in the  
32 patient;

1 (b) providing an inoculum comprising isolated bone marrow  
2 and/or stem-cell enriched peripheral blood cells of the donor  
3 treated with a T-cell depleting effective amount of a  
4 recombinant immunotoxin polypeptide or its pharmaceutically  
5 acceptable salt according to claim 1;

6 (c) introducing the inoculum into the patient; and

7 (d) transplanting the donor cells, tissue or organ into the  
8 patient.

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10 28. A method according to claim 21 comprising co-administering  
11 the recombinant immunotoxin polypeptide or its pharmaceutically  
12 acceptable salt with at least one other pharmaceutical agent  
13 selected from cyclosporin A, rapamycin, 40-O-(2-hydroxy)ethyl  
14 rapamycin (RAD), FK-506, mycophenolic acid, mycophenolate mofetil  
15 (MMF), cyclophosphamide, azathioprene, leflunomide, mizoribine, a  
16 deoxyspergualine compound or derivative or analog, 2-amino-2-[2-  
17 (4-octylphenyl)ethyl]propane-1,3-diol, corticosteroids, anti-LFA-  
18 1 and anti-ICAM antibodies, and other antibodies that prevent co-  
19 stimulation of T cells.

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21 29. A pharmaceutical composition comprising a recombinant  
22 immunotoxin polypeptide or its pharmaceutically acceptable salt  
23 according to claim 1 in a pharmaceutically acceptable carrier.

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